Is Redox Regulation of Protein Arginine Methyltransferase 6 Cysteine Mediated?

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Abstract

Protein arginine methyl transferases (PRMTs) are a family of proteins that modify protein targets though the addition of a methyl group (Figure 1). PRMTs play roles in DNA repair, cancer, heart disease, epigenetic programming, and many other cellular functions. The biochemical mechanisms that govern PRMT regulation are still poorly understood. Additionally, arginine methylation appears to be a permanent modification, as no arginine demethylases have been identified. This implicates a further importance of proper PRMT regulation. The Hevel lab has recently characterized an intrinsic redox dependent modulator of PRMT1 activity. We showed that cysteines 101 and 208 of PRMT1 sense redox conditions, which gives rise to altered enzymatic activity. Interestingly, we have also observed redox sensitivity in PRMT6, a protein highly homologous to PRMT1. Comparisons of oxidized and reduced PRMT6 crystal structures show that cysteine residues (53 and 232) non-homologous to those identified in PRMT1 form a disulfide bond, a type of oxidized cysteine (Figure 2). This implies that the mechanism of redox regulation in PRMT6 may be different than in PRMT1. We hypothesize that one or several of these cysteine residues are responsible for the redox induced modulation of PRMT6 activity.

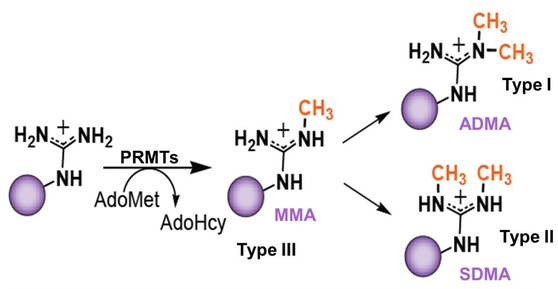

Figure 1: PRMT catalyzed reaction. target protein substrate (left), methylated protein product (right).

Methods & Results

To test this hypothesis, we will 1) purify recombinant wild type and cysteineless PRMT6 proteins using Nickel affinity chromatography, and 2) measure the activity for these proteins under both oxidative and reductive conditions. If our hypothesis is correct, the activity of cysteineless PRMT6 will be unaffected by reductive/oxidative conditions.

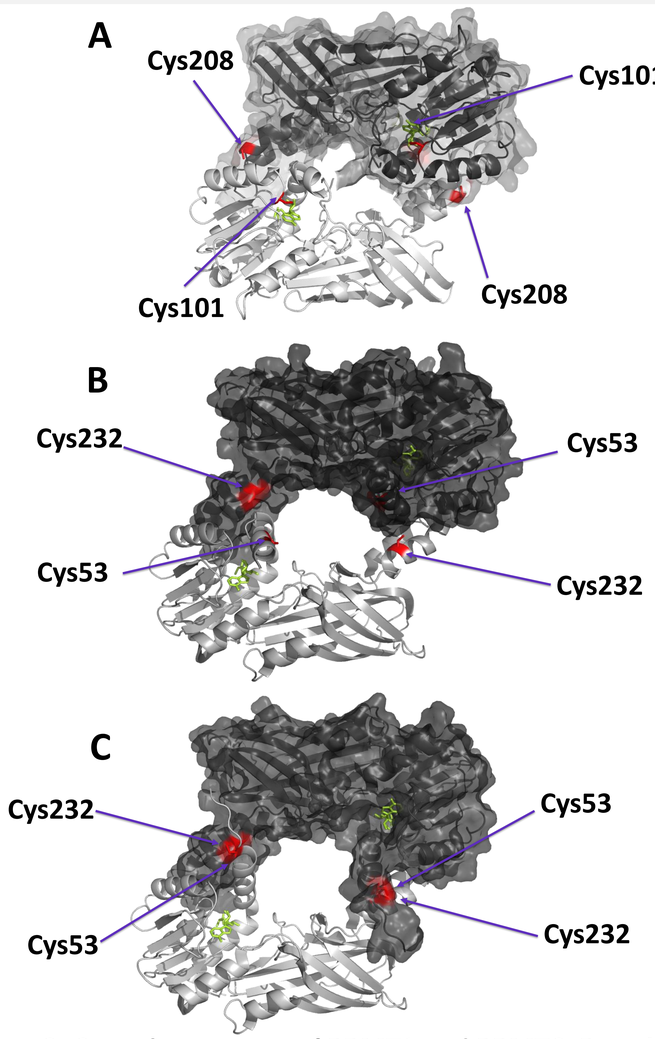

Figure 2: Crystal structures of PRMT1 and PRMT6. Putative redox active cysteine residues are shown in red. A: PRMT1. B: Reduced PRMT6. C: Oxidized PRMT6.

Expression & Purification

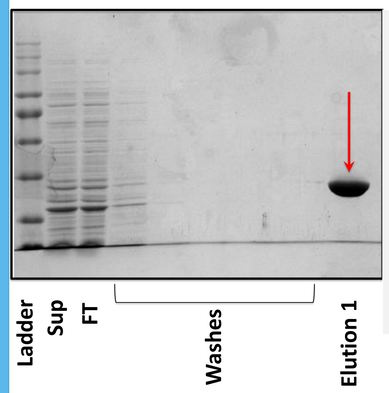

Figure 3: Coomassie staine of Δcys PRMT6 purification fractions. This procedure yields Δcys PRMT6 of adequate purity, however the wild type PRMT6 was not purified adequately using the same protocol.

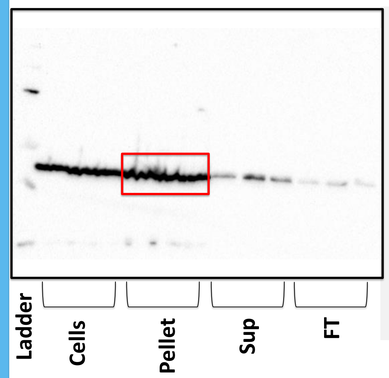

Figure 4. A western blot (anti-his) was utilized in order to determine potential problems with wild type expression. This experiment identified a relatively high concentration of protein within the cell pellet (red).

Future Directions

Continue to refine the PRMT6 expression protocol for high levels of soluble, active protein. Assay both wild type and cysteineless (mutant) forms of PRMT6 under oxidized and reduced conditions.

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